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Proposal for a New Score-Based Approach To Improve Efficiency of Diagnostic Laboratory Workflow for Acute Bacterial Meningitis in Adults

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Microbiological tests on cerebrospinal fluid (CSF) utilize a common urgent-care procedure that does not take into account the chemical and cytological characteristics of the CSF, resulting sometimes in an unnecessary use of human and diagnostic resources. The aim of this study was to retrospectively validate a simple scoring system (bacterial meningitis-Careggi score [BM-CASCO]) based on blood and CSF sample chemical/cytological parameters for evaluating the probability of acute bacterial meningitis (ABM) in adults. BM-CASCO (range, 0 to 6) was defined by the following parameters: CSF cell count, CSF protein levels, CSF lactate levels, CSF glucose-to-serum glucose ratio, and peripheral neutrophil count. BM-CASCO was retrospectively calculated for 784 cases of suspected ABM in adult subjects observed during a four-and-a-half-year-period (2010 to 2014) at the emergency department (ED) of a large tertiary-care teaching hospital in Italy. Among the 28 confirmed ABM cases (3.5%), *Streptococcus pneumoniae* was the most frequent cause (16 cases). All ABM cases showed a BM-CASCO value of ≥3. Most negative cases (591/756) exhibited a BM-CASCO value of ≤1, which was adopted in our laboratory as a cutoff to not proceed with urgent microbiological analysis of CSF in cases of suspected ABM in adults. During a subsequent 1-year follow-up, the introduction of the BM-CASCO in the diagnostic workflow of ABM in adults resulted in a significant decrease in unnecessary microbiological analysis, with no false negatives. In conclusion, BM-CASCO appears to be an accurate and simple scoring system for optimization of the microbiological diagnostic workflow of ABM in adults.

Cases of acute bacterial meningitis (ABM) require prompt diagnosis and treatment due to significant mortality rates (1, 2). A delay in starting appropriate therapy may worsen the prognosis (1). Recently, the epidemiology of ABM has shifted to older age groups due to the increasing child vaccination rates against the most common meningeal pathogens (3, 4). *Streptococcus pneumoniae* is currently the leading cause of ABM in adults and is associated with a 17 to 30% mortality rate (2–4).

Diagnosis of ABM at the time of clinical presentation is often difficult and requires laboratory investigation of cerebrospinal fluid (CSF) specimens. A Gram stain of the CSF sediment is a widely used test for the rapid detection of bacteria but may suffer from low sensitivity (5). When evaluated on adult subjects not previously treated with antibiotics, the CSF Gram stain has been reported to have a sensitivity between 60% and 92% (6). Sensitivity can be increased by molecular tests, such as real-time PCR (RT-PCR), but these tests are still not widespread due to their high cost and the need for expensive equipment and experienced laboratory personnel. Bacterial culture remains the gold standard for microbiological diagnosis of ABM, but it requires longer times and suffers from reduced sensitivity in cases involving previous antimicrobial chemotherapy (6). In addition, biochemical and cytological alterations of CSF may help in the diagnostic process of ABM. The most common alterations include polymorphonuclear leukocytosis, decreased glucose concentration, and increased protein and lactate concentrations (5, 6).

Lumbar puncture (LP) for CSF examination represents a common procedure performed in emergency departments (EDs) when dealing with subjects with suspected ABM (7). In the current diagnostic workflow, the microbiological analysis of CSF samples (Gram stain, culture and, possibly, RT-PCR for common bacterial pathogens) is usually carried out as an urgent procedure with all CSF samples, regardless of the biochemical and cytological parameters of CSF.

In this study, we evaluated the performance of a simple scoring system (bacterial meningitis-Careggi score [BM-CASCO]), based on biochemical and cytological CSF and blood parameters, that could be used for triage of suspected cases of ABM in adults to be subjected to urgent microbiological workup of CSF, eliminating useless tests and sparing human resources.

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TABLE 1 Values of blood and CSF parameters used to calculate the BM-CASCO[a]

Blood/CSF parameters	Cutoff value	BM-CASCO score
CSF cell count	≤50 cells/μl	0
	>50 cells/μl	2
CSF protein concn	≤80 mg/dl	0
	>80 mg/dl	1
CSF lactate concn	≤35 mg/dl	0
	>35 mg/dl	1
CSF glucose-to-serum glucose ratio	≥45%	0
	<45%	1
Peripheral neutrophil count	≤10,000 cells/μl	0
	>10,000 cells/μl	1

[a] The score results from the sum of all individual values and can range between 0 and 6.

MATERIALS AND METHODS

Data sources and study period. The hospital discharge register and medical records of all patients evaluated in the ED between 1 January 2010 and 31 May 2014, and who were considered suspected cases of ABM, were examined for clinical, laboratory, and microbiological data for the retrospective evaluation of BM-CASCO.

Definitions. A suspected case of adult ABM was defined as any case, age >18 years, admitted to the ED and subjected to LP for CSF analysis to confirm a diagnosis of ABM. A confirmed case of ABM was defined as a clinically compatible case with a positive CSF culture and/or RT-PCR test for a bacterial pathogen in a subject age >18 years. A contaminant was defined as a bacterial isolate from CSF in a case that was not compatible with a diagnosis of ABM according to the clinical presentation and other laboratory data.

Data collection and analysis. We retrospectively collected the following data from clinical and laboratory records: date of birth, gender, results of CSF Gram stain, results of CSF bacteriological cultures, results of CSF molecular analysis, peripheral blood and CSF leukocyte (WBC) count, CSF glucose-to-serum glucose ratio, CSF protein concentration, CSF lactate concentration, and diagnosis at discharge. Molecular analysis of CSF for bacterial pathogens was carried out only in selected cases, when the results of Gram stain were considered not informative, at the discretion of the clinical microbiologist in charge of the case. The data were entered into an Excel data sheet and analyzed with IBM SPSS Statistics (Armonk, NY, USA). An evaluation of sensitivity, specificity, and negative predictive value of the BM-CASCO was carried out as described previously (8). Ethical approval was not needed because the study used fully anonymized observational data that were obtained as part of an assessment of routine clinical service.

Design of the score. The score was designed based on clinical experience and a review of the literature (5–7, 9). BM-CASCO (range, 0 to 6) was defined as the sum of a score attributed to the following parameters: 2 points for a CSF leukocyte count of >50 cells/μl and 1 point for a CSF protein concentration of >80 mg/dl, a CSF lactate concentration of >35 mg/dl, a CSF glucose-to-serum glucose ratio of <45%, or a peripheral neutrophil leukocyte count of >10,000 cells/μl (Table 1).

Evaluation of the implementation phase. In the period of 1 June 2014 to 31 May 2015, the BM-CASCO was applied in our laboratory diagnostic workflow for adult patients admitted to the ED with a suspected diagnosis of ABM. Following this new procedure, microbiological analysis of CSF was no longer performed when the BM-CASCO was ≤1, unless specifically requested by the ED physician, who was left with the option to override the procedure based on clinical judgment.

RESULTS

Between 1 January 2010 and 31 May 2014, a total of 813 suspected cases (397 female and 416 male) of ABM age >18 years were evaluated at the ED of Careggi University Hospital, Florence, Italy. During the same period, the ED evaluated about 150 patients/day. Twenty-nine cases were excluded from subsequent analysis due to incomplete clinical data. All of them had a negative CSF culture.

Among the 784 cases included in the analysis, 55 (7.0%) had a CSF-positive result for bacteria (54 yielded positive cultures, and one was positive by RT-PCR test only). Of these positives, 27 were interpreted to be contaminations, considering the clinical and laboratory findings. In those cases, the most common bacterial species were coagulase-negative staphylococci (Table 2). The overall rate of contaminated CSF was 3.4%. The remaining 28 cases with a CSF-positive result for bacteria were confirmed cases of ABM according to clinical and laboratory findings. In one case, only the molecular test was positive, likely because of previous antimicrobial treatment. The most common pathogen was *S. pneumoniae* (Table 2). These 28 ABM cases were considered to be true positives for an evaluation of the BM-CASCO.

All cases of confirmed ABM showed a BM-CASCO value of

TABLE 2 Distribution of the BM-CASCO among 784 cases of suspect ABM evaluated at the ED of Careggi University Hospital from 1 January 2010 to 31 May 2014

		No. (%) with BM-CASCO value:							
Definitive diagnosis	CSF culture result	0	1	2	3	4	5	6	Total (no. [%])
No ABM	Negative	384	185	75	44	32	4	5	729 (93.0)
	Contaminated[a]	19	3	1	1	3	0	0	27 (3.45)
Confirmed ABM	Positive[b]	0	0	0	2	1	5	19	27 (3.45)
	Negative[c]	0	0	0	0	0	0	1	1 (0.1)
Total		403 (51.4)	188 (24.0)	76 (9.7)	47 (6.0)	36 (4.6)	9 (1.1)	25 (3.2)	784 (100)

[a] Bacterial species cultured in these cases included *Staphylococcus epidermidis* ($n = 5$), *Staphylococcus warneri* ($n = 4$), *Staphylococcus hominis* ($n = 4$), *Staphylococcus capitis* ($n = 3$), *Staphylococcus auricularis* ($n = 1$), *Staphylococcus cohnii* ($n = 1$), *Staphylococcus haemolyticus* ($n = 1$), *Streptococcus mitis* ($n = 1$), *Streptococcus* spp. (alpha-hemolytic) ($n = 1$), *Micrococcus luteus* ($n = 2$), *Corynebacterium minutissimum* ($n = 1$), *Bacillus* spp. ($n = 1$), *Acinetobacter lwoffii* ($n = 1$), and *Escherichia coli* ($n = 1$). In all these cases, the bacterial isolates were considered to be contaminants based on clinical and laboratory findings. The *E. coli* strain, in particular, was grown from the CSF specimen from a 60-year-old woman with a history of chronic migraine, who was discharged the day after admission with a diagnosis of chronic migraine and was not given any antimicrobial treatment.

[b] Bacterial species cultured in these cases included *S. pneumoniae* ($n = 15$), *Staphylococcus aureus* ($n = 4$), *Neisseria meningitidis* ($n = 4$), *Streptococcus agalactiae* ($n = 1$), *Haemophilus influenzae* ($n = 1$), and *Listeria monocytogenes* ($n = 2$).

[c] This case was positive for *S. pneumoniae* with the RT-PCR test.

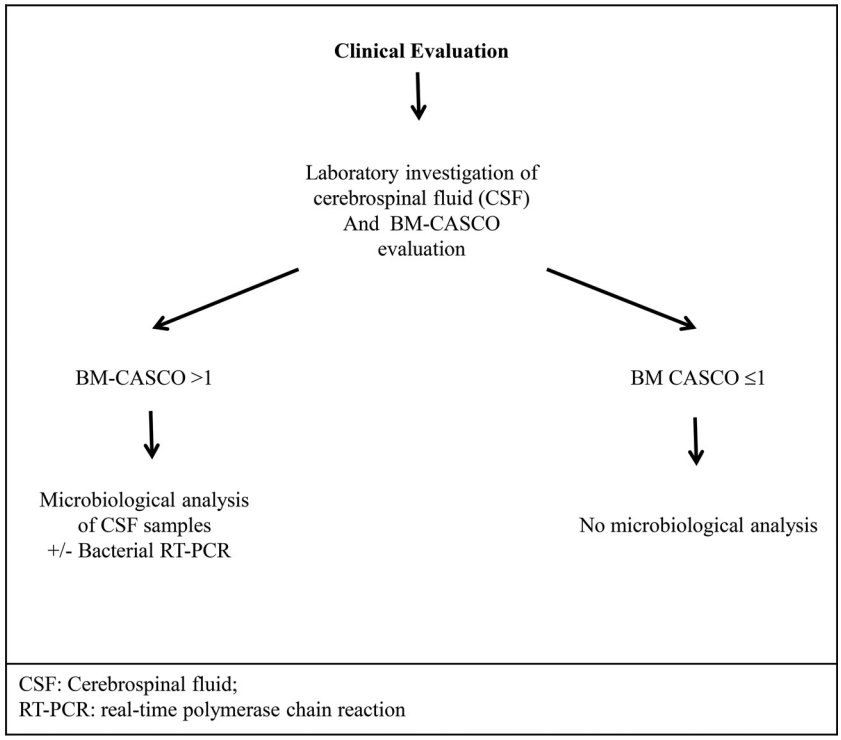

FIG 1 Diagnostic laboratory workflow for ABM in adults.

≥3, with median and mean values of 6 and 5.2, respectively (Table 2). Considering all evaluable samples, 591/784 (75.4%) yielded a BM-CASCO value of ≤1 (Table 2).

When applying a BM-CASCO value of ≤1 as a cutoff to exclude the diagnosis of ABM, the sensitivity and negative predictive value (NPV) were 100% (95% confidence interval [CI], 87.6% to 100%) and 100% (95% CI, 99% to 100%), respectively, while specificity was 78.1% (95% CI, 75% to 81.7%).

Based on these results, the BM-CASCO was introduced in the diagnostic workflow of ABM in adults admitted to the ED of our hospital. Altogether, during the period of 1 June 2014 to 31 May 2015, a total of 215 CSF specimens were obtained from adults admitted to the ED with diagnosis of suspected ABM. Of these, 152 (70%) yielded a BM-CASCO value of ≤1 and were not subjected to further microbiological analysis. None of these cases were eventually diagnosed as ABM cases according to the subsequent clinical follow-up. Among the 63 cases with a BM-CASCO value of >1 who were subjected to further microbiological analysis, five (7.2%) were eventually confirmed to be ABM cases. Considering the subset of 120 CSF specimens received during on-call shifts of the clinical microbiology service, 96 of them (80%) exhibited a BM-CASCO value of ≤1 and did not require activation of the on-call procedure.

DISCUSSION

Although the number of definite indications for LP has decreased with the introduction of streamlined neuroimaging procedures, LP is commonly performed in EDs in adult subjects with some combination of fever, altered mental status, headache, or meningeal signs to confirm diagnosis of ABM, and CSF sample analysis is usually performed in accordance with urgent procedures, including cell counts, biochemistry, and microbiological evaluation (7, 10, 11). Our clinical microbiology laboratory was used to perform CSF Gram stain and bacterial culture (and also RT-PCR in selected cases) as urgent exams, on a 24-hours-a-day, 7-days-a-week schedule, on all CSF specimens collected in the ED from cases of suspected ABM.

A retrospective review of the laboratory reports of our hospital, however, showed that 75% of CSF microbiological analyses were performed on samples with no or minimal changes in white cell counts and biochemical parameters, and that only 3.5% of cases of suspected ABM observed in the ED were eventually confirmed. These findings prompted us to elaborate a CSF processing strategy to limit urgent and inappropriate microbiological tests while maintaining an optimal performance for diagnosing ABM. The strategy was based on a simple score, the BM-CASCO, calculated from a set of five parameters derived from the initial clinical chemistry workup that were chosen by a review of the literature (5–7, 9). Although previous scoring systems have been proposed to distinguish bacterial from viral meningitis (12–16), to the best of our knowledge, this is the first scoring system based only on clinical chemistry data for triage of suspected cases of ABM in adults to be subjected to further microbiological analysis of CSF. As a matter of fact, differently from other scores (14–16), neither microbiological (e.g., CSF Gram stain) nor clinical rules were taken into account. Gram stain, which is usually present in some previously reported score systems, has been substituted by an easily replicable laboratory parameter, such as measurement of CSF lactate levels. This parameter was shown in a recent meta-analysis to discriminate well between bacterial and aseptic meningitis (9).

Considering the objective, the BM-CASCO prediction rules were designed to exhibit maximal sensitivity to avoid the misclas-

sification of even a single ABM case. Although all CSF analyses for suspected ABM cases from a four-and-a-half-year period were included, the main limitation of our study is represented by the retrospective design. Taking into account this limitation, we applied a conservative cutoff of ≤1 for the BM-CASCO, even though all cases of confirmed ABM showed a score of ≥3.

In virtue of the 100% NPV with a BM-CASCO value of ≤1, a new diagnostic procedure for CSF workup has been applied in our laboratory, in agreement with recommendations of ED physicians and infectious diseases specialists, since 1 June 2014. In accordance with this new procedure, microbiological analysis of CSF is performed only when the BM-CASCO value is >1. The ED physicians, however, are aware that ABM cases with no abnormalities in the initial CSF testing are possible, although extremely rare, with specific clinical conditions (e.g., congenital or acquired immune deficiencies in host defense mechanisms, strong epidemiological context, neurosurgery, prior antibiotic therapy, etc.) (17–20); they are allowed to override the new procedure and request further urgent microbiological analysis of CSF, irrespective of the BM-CASCO (Fig. 1).

In fact, after a 1-year follow-up since the introduction of the new procedure based on BM-CASCO, we were able to document an 80% reduction in urgent microbiological test performed on CSF specimens referred by the ED in cases of suspected ABM in adults, with a significant sparing of human resources either during working hours or during the on-call shifts.

In an era of financial constraints, budget planning is mandatory, and costs related to unnecessary tests and emergency calls of highly skilled staff should be optimized. Carrying out CSF analysis as an urgent procedure regardless of the biochemical and cytological parameters generates a useless waste of human and material resources and, in cases of contaminated samples, might even provide misleading results. With this perspective, BM-CASCO could represent a valid tool for an appropriate laboratory procedure for CSF analysis in case of adult patients with suspected ABM, eliminating useless tests and correctly allocating human resources.

However, considering that bacterial meningitis with no abnormalities in initial CSF testing is possible, although extremely rare, repeat CSF analysis should be considered, and antimicrobial therapy must be started immediately in the presence of any signs of sepsis or meningitis (7, 18–20).

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